# ADJUVANTED VACCINES WITH NON-VIRION ANTIGENS PREPARED FROM INFLUENZA VIRUSES GROWN IN CELL CULTURE

This application is a national stage application of PCT/GB2006/004128 filed Nov. 6, 2006, which claims the benefit of Serial No. 60/734,026 filed Nov. 4, 2005 and Serial No. 60/735,658 filed Nov. 11, 2005. Each of these applications is incorporated herein by reference in its entirety.

All documents cited herein are incorporated by reference in their entirety.

## TECHNICAL FIELD

This invention is in the field of adjuvanted vaccines for protecting against influenza virus infection.

## BACKGROUND ART

The current standard method for influenza virus growth in vaccine manufacture uses embryonated SPF hen eggs, with virus being purified from the egg contents (allantoic fluid). More recently, however, viruses have been grown in cell culture, and this method has the potential for producing larger quantities of antigen in a shorter time. In addition, it offers the ability to produce viruses which, due to their avian pathogenicity, cannot be grown in eggs.

Reference 1, from scientists at Baxter, reports a comparison of trivalent whole-virion vaccines (WVV) prepared from viruses grown either on eggs or on Vero cells. The two vaccines were compared for their ability to induce humoral and cell-mediated immunity. The authors reported that the immunogenicity of the Vero-derived vaccine was comparable to that of the egg-derived vaccine, but that the Vero-derived vaccine was superior in terms of T cell responses. T cell responses are reported to be more resistant than antibody responses to seasonal influenza virus antigenic drift, thereby improving year-to-year immunity.

With these encouraging results, Baxter continued to develop the Vero-derived product, under the trade name PREFLUCEL™. In December 2004, however, Baxter suspended its Phase II/III clinical study because the rate of fever and associated symptoms was higher than seen with existing vaccines.

Thus there remains a need for a safe and effective vaccine based on influenza virus grown in cell culture rather than in eggs.

## DISCLOSURE OF THE INVENTION

Various forms of influenza virus vaccine are currently available (e.g. see chapters 17 & 18 of reference 2). Many vaccines are based on live virus or inactivated virus, with inactivated vaccines being based on whole virions, 'split' virions, or on purified surface antigens (including hemagglutinin and neuraminidase). The failed PREFLUCEL™ product used whole influenza virions.

The use of whole virions may be associated with increased reactogenicity [3]. To avoid the reactogenic problems seen with the PREFLUCEL™ product, the invention does not use a whole virion antigen i.e. it uses a non-virion antigen (e.g. a split virion, or purified surface antigens). The antigens are derived from virus grown in cell culture. While T cell responses were reported [1] to be enhanced when using whole virions grown in cell culture, however, the data herein show only modest T cell responses when using non-virion antigens. To provide enhanced T cell responses, therefore, the invention combines the non-virion antigens with an adjuvant.



The PREFLUCEL™ product did not include an adjuvant, and adding adjuvants to influenza vaccines has previously been linked to potential hypersensitivity. For example, reference 4 reports that an alum-adjuvanted influenza vaccine could sensitize guinea pigs, while unadjuvanted vaccine did not, and that the anaphylactogenic activity of egg proteins was significantly increased by the adjuvant. Similarly, reference 5 reported that adsorption of influenza virus antigen to aluminum salts led to earlier ovalbumin sensitization compared to unadjuvanted antigen. Furthermore, reference 6 reports that animals who previously received alum-adjuvanted ovalbumin showed an exacerbated allergic response during the early stages of an influenza virus infection. Hypersensitivity to vaccine components is a particular problem for influenza vaccines, as they are usually administered every year. By avoiding an egg-based system for viral growth, the invention also advantageously avoids any ovalbumin-linked concerns, which could become more apparent as influenza vaccination becomes more widespread (e.g. as immunization is extended to patient groups who have not previously been indicated for vaccination, and as the proportion of patients who are immunized in indicated target groups increases).

Thus the invention provides an immunogenic composition comprising: (i) a non-virion influenza virus antigen, prepared from a virus grown in cell culture; and (ii) an adjuvant.

The invention also provides a method for preparing an immunogenic composition comprising the steps of combining: (i) a non-virion influenza virus antigen prepared from a virus grown in cell culture; and (ii) an adjuvant.

The invention also provides a kit comprising: (i) a first kit component comprising a non-virion influenza virus antigen prepared from a virus grown in cell culture; and (ii) a second kit component comprising an adjuvant.

The influenza virus antigen typically comprises an influenza virus haemagglutinin. The adjuvant is preferably an oil-in-water emulsion adjuvant, such as MF59, and more preferably does not include any aluminum salt(s). Oil-in-water emulsions have been found to enhance influenza-specific T cell responses, and they can also enhance memory B cell responses. In addition, they can improve cross-reactivity against heterovariant influenza strains, such that a vaccine may induce protective immunity even if the vaccine strain does not match the circulating strain.

The use of adjuvants with influenza vaccines has been described before. In references 7 & 8, aluminum hydroxide was used to adjuvant Vero-derived whole virion vaccines. In reference 9, a mixture of aluminum hydroxide and aluminum phosphate was used to adjuvant egg-derived vaccines, with the preferred vaccines being egg-produced monovalent vaccines against pandemic strains. In reference 57, aluminum hydroxide was used to adjuvant MDCK-derived inactivated virions. Reference 10, for instance in example 7, suggests using adjuvants with inactivated whole equine influenza viruses. Reference 11 discloses, for instance in example 5, using aluminum hydroxide with inactivated virus grown on chicken embryo cells. In example 2 of reference 12, various different adjuvants were used with a trivalent egg-derived split vaccine. In reference 13, aluminum salts were used to adjuvant monovalent egg-derived whole virion vaccines. In most of these prior art cases, however, adjuvant was used with a whole-virion vaccine, and was not used with an antigen derived from virus grown in cell culture. More-